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Notable Hesperiidae collected by Kilian Roever in Arizona, USA

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ABSTRACT. Genomic analysis of Hesperiidae specimens collected by Kilian Roever in Arizona, USA, and deposited in the McGuire Center for Lepidoptera and Biodiversity (Florida Museum of Natural History, Gainesville, FL, USA) confirms several published state species records, reveals that *Cecropterus* (*Murgaria*) *markwalkeri* Grishin, 2023, *Telegonus* (*Telegonus*) *misitra* (Plötz, 1881), and *Epargyreus cruza* Evans, 1952 have been found in Arizona with sequenced specimens representing first confirmed USA records of these three species, and allows us to discover new taxa *Cecropterus* (*Murgaria*) *roeveri* Grishin, **sp. n.** and *Lerema* (*Lerema*) *ochrius occidus* Grishin, **ssp. n**. This study underscores the value of extensive collecting and careful specimen preservation for research.

Key words: taxonomy, classification, genomics, phylogeny, biodiversity.

ZooBank registration: http://zoobank.org/4F75FF52-85A5-494B-8757-52A732DCB578

INTRODUCTION AND METHODS

In contrast to photographs, which are only as good as the pose and our ability to recognize a species from its appearance, collected and carefully preserved specimens enable future studies using various methods. Dissection of genitalia is essential for the identification of many Hesperiidae Latreille, 1809 that cannot be confidently told apart by their wing patterns (Evans 1951, 1952, 1953, 1955). Recently, DNA-based approaches, in particular, those deriving power from whole genome sequencing, offer a way of confident identification, species discovery, and even determining collecting localities by genomic comparison (Cong et al. 2021). We use these methods to study butterflies, and this work builds upon our previous publications, following similar principles and employing the same techniques (Cong et al. 2019a, b; Li et al. 2019; Zhang et al. 2019a–d; Cong et al. 2020; Zhang et al. 2020; Cong et al. 2021; Zhang et al. 2021; Robbins et al. 2022; Zhang et al. 2022a, b; 2023a, c–g; 2024a–c).

Here, we apply whole genome sequencing and analysis to specimens of rarely observed species reaching their northern distribution limits in the southern USA. We focus on specimens collected by Kilian Roever in southeastern Arizona and deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA (MGCL). A leg was sampled from each specimen for DNA extraction and sequencing. The methods were exactly as those described in our latest two publications (Zhang et al. 2024a, b), and the introduction to Zhang et al. (2024b) summarizes general principles we use for species delineation. Here, for brevity, we show only two genomic trees in each figure: one nuclear tree (either constructed from protein-coding genes in autosomes, or the Z chromosome when it reveals better separation between species) and the mitochondrial genome tree.

Sections of this work are arranged in taxonomic order deduced from genome-scale phylogeny complemented by phenotypic considerations. For the new taxa, in addition to brief phenotypic diagnoses frequently accompanied by references that discuss and illustrate morphological characters in greater detail, we provide diagnostic DNA characters in the nuclear genome and in the COI barcode. DNA characters are found in nuclear protein-coding regions using our previously developed procedure (see SI Appendix to Li et al. 2019). The logic behind the character selection was described in Cong et al. (2019b) and is aimed at finding more robust characters likely to stand when additional specimens and species are sequenced. The character states are given in species diagnoses as abbreviations. E.g., aly728.44.1:G672C means position 672 in exon 1 of gene 44 from scaffold 728 of the *Cecropterus lyciades* (Geyer, 1832) (aly, because this species was formerly in the genus *Achalarus* Scudder, 1872) reference genome (Shen et al. 2017) is C, changed from G in the ancestor. When characters are given for the sister clade of the diagnosed taxon, the following notation is used: aly5294.20.2:A548A (not C), which means that position 548 in exon 2 of gene 20 on scaffold 5294 is occupied by the ancestral base pair A, which was changed to C in the sister clade (so it is not C in the diagnosed taxon). The same notation is used for COI barcode characters, but without a prefix ending with ':'. The sequences of exons from the reference genome with the positions used as character states highlighted in green are in the supplemental file deposited at < https://osf.io/sp7fn/ >. This link to the DNA sequences accessible from this publication ensures that DNA characters given in the diagnoses can be readily associated with actual sequences.

Whole genome shotgun datasets we obtained and used in this work are available from the NCBI database < https://www.ncbi.nlm.nih.gov/ > as BioProject PRJNA1243725 and BioSample entries of the project contain the locality and other collection data of the sequenced specimens shown in the trees. For each specimen in tree figures, the following information is provided (separated by '|'): taxon name with comments in square brackets, DNA sample code, type status, general locality, and year of collection ('old' if not dated and likely collected 100–150 years ago). Type status abbreviations are: HT holotype, LT lectotype, ST syntype, ?ST possible syntype, NT neotype, PT paratype, PLT paralectotype; and if a synonym name is given (in parenthesis, preceded by '='), type status refers to the synonym. COI barcode sequences reported here have been deposited in GenBank with accessions PV408305 and PV408306. Abbreviations or acronyms for collections are listed in the acknowledgments section.

RESULTS AND DISCUSSION

Specimens of species rarely encountered in the USA were selected for genomic sequencing from the Roever collection, now in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA. Genomic comparison with other sequenced specimens, including many primary types, provided identification of these species, mostly confirming previously published phenotype-based identifications (Bailowitz and Brock 1991, 2022) corrected for the updates in taxonomy since then (Warren et al. 2024). These advances in our understanding of taxonomy and recent descriptions of new species (Zhang et al. 2023b, f) resulted in three confirmed new US records of butterflies: *Cecropterus* (*Murgaria*) *markwalkeri* Grishin, 2023 (type locality in Mexico: Sonora), *Telegonus* (*Telegonus*) *misitra* (Plötz, 1881) (type locality in Mexico), and *Epargyreus cruza* Evans, 1952 (type locality in Mexico: Veracruz). Finally, a new species and a new subspecies were discovered and are described here. Details are given for each taxon below.

Cecropterus (*Murgaria*) *markwalkeri* Grishin, 2023

As hypothesized in Zhang et al. (2024b), we confirm by genomic analysis that at least some records referred to as *Cecropterus albociliatus* (Mabille, 1877) (type locality in Colombia, Panama, Guatemala) from specimens collected by Kilian Roever in southeastern Arizona (Bailowitz and Brock 1991, 2022) are *Cecropterus* (*Murgaria*) *markwalkeri* Grishin, 2023 (type locality in Mexico: Sonora) (Fig. 1 shaded yellow). We sequenced the following three specimens from USA, Arizona, Santa Cruz Co.: NVG-

24074G12 Mt. Hopkins Rd., 8 mi E of Amado, 18-Jun-1978 (Fig. 2a, genitalia Fig. 3); NVG-24074H01 Santa Cruz Mts., Temporal Gulch, 18-Jun-1978 (Fig. 2b); and NVG-24074H02 Sycamore Canyon near Ruby, 26-Aug-1988.

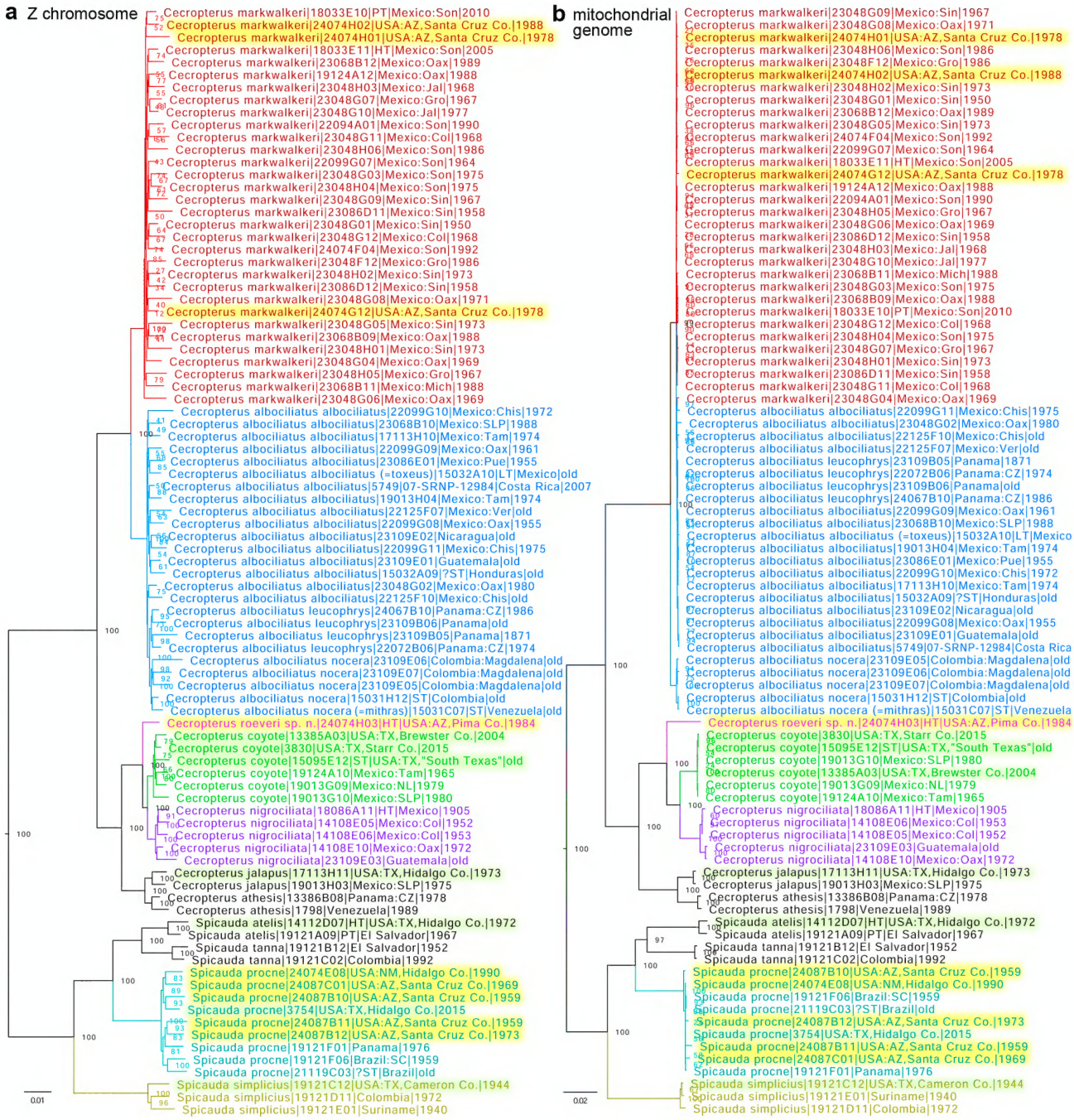

Fig. 1. Phylogenetic trees of selected *Cecropterus* (*Murgaria*) and *Spicauda* species inferred from protein-coding regions in **a)** the Z chromosome, based on 361,947 positions, and **b)** the mitochondrial genome: *C. markwalkeri* (red), *C. albociliatus* (blue), *C. roeveri* **sp. n.** (magenta), *C. coyote* (green), *C. nigrociliata* (purple), *S. procne* (cyan), and *S. simplicius* (olive). Ultrafast bootstrap (Minh et al. 2013) values are shown at nodes. Labels of specimens from Arizona and Texas are highlighted in yellow and green, respectively. For each specimen, the name adopted in this work is given first, supplemented with the DNA Sample number, type status (see Introduction for abbreviations), general locality, and collection year. Synonyms are given in parentheses preceded by "=" (in subsequent figures). The type status refers to this synonym if the synonym name is provided. The same notations are used throughout this work in other figures showing phylogenetic trees.

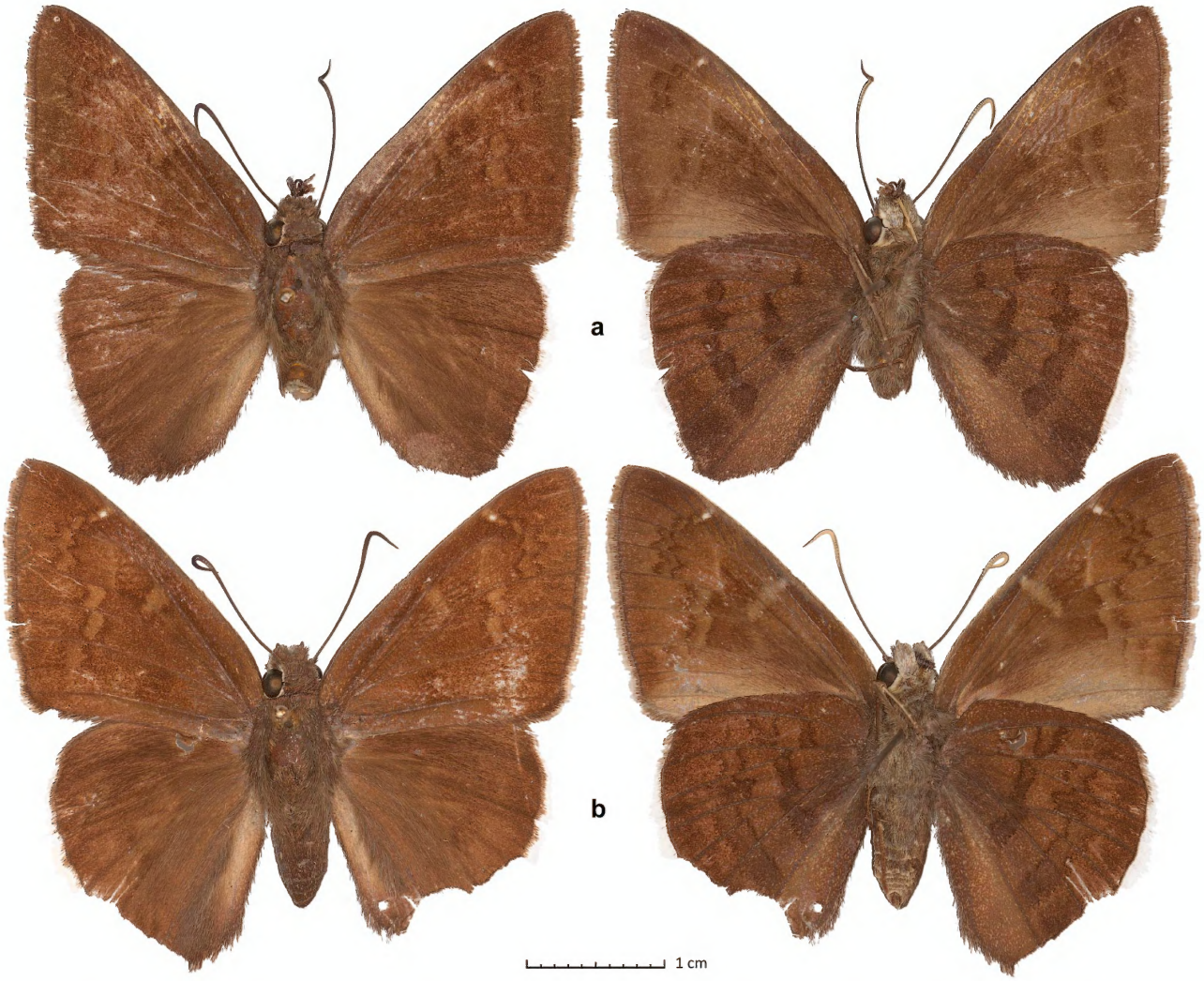

Fig. 2. *Cecropterus* (*Murgaria*) *markwalkeri* from USA, Arizona in dorsal (left) and ventral (right) views, data in text: **a)** NVG-24074G12 and **b)** NVG-24074H01.

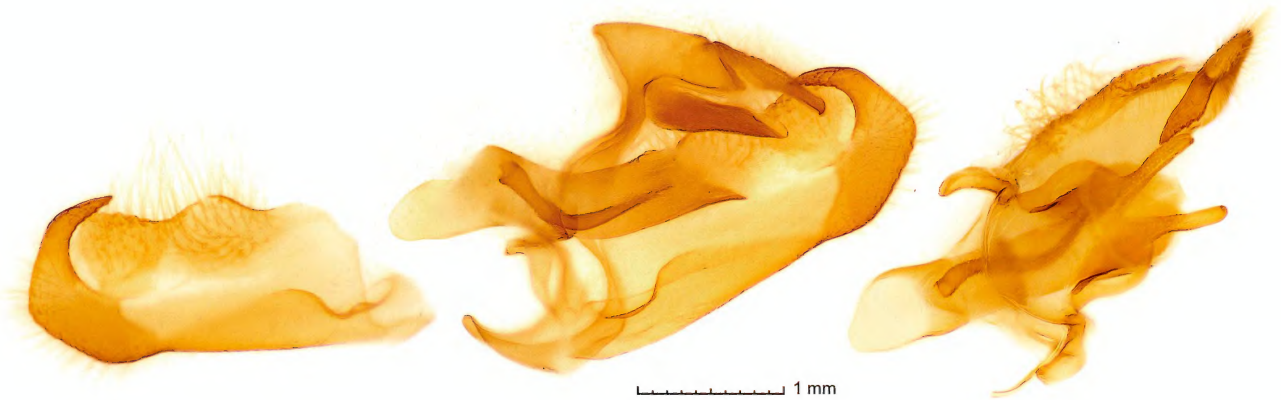

Fig. 3. Male genitalia of *Cecropterus markwalkeri* NVG-24074G12 from AZ: left valva in right lateral view (left) and the rest of the genitalia in left lateral (middle) and dorsal (right) views; unnumbered dissection by Kilian Roever or his helpers.

Cecropterus (*Murgaria*) *roeveri* Grishin, new species

http://zoobank.org/27B71385-5F34-426D-8446-BBBE8549DD27

(Figs. 1 part, 4, 5a)

Definition and diagnosis. A specimen of *Cecropterus* (*Murgaria*) E. Watson, 1893 (type species *Telegonus albociliatus* Mabille, 1877) collected by Kilian Roever in southeastern Arizona (Fig. 1 magenta) is sister to both *Cecropterus* (*Murgaria*) *coyote* (Skinner, 1892) (type locality in USA: Southern Texas) (Fig. 1 green) and *Cecropterus* (*Murgaria*) *nigrociliata* (Mabille & Boullet, 1912) (type locality in Mexico) (Fig. 1 purple) in the Z chromosome and the mitochondrial genome trees, and is genetically differentiated from them at the species level, e.g., their COI barcodes differ by 3.3% (22 bp) from *C. coyote* and by 2.7% (18 bp) from *C. nigrociliata*, and therefore represents a new species. This new species keys to "*Achalarus toxeus*" (C.17.4) in Evans (1952), who misidentified *Aethilla toxeus* Plötz, 1882 (type locality in Mexico), currently treated as a junior subjective synonym of *Cecropterus* (*Murgaria*) *albociliatus albociliatus* (Mabille, 1877) (type locality in Colombia, Panama, and Guatemala) (Zhang et al. 2023f). Evans's "*A. toxeus*" corresponds to the species group with *C. coyote* and *C. nigrociliata*. The new species differs from others by its male genitalia (Fig. 5a) with harpe narrowing in the middle and turning posterodorsad and narrowing to a point directed anterodorsad (harpe of both *C. coyote* and *C. nigrociliata* (Fig. 5b) is blade-like, relatively straight, terminally rounded and dorsally serrated, broader and shorter in *C. coyote* and narrower and longer in *C. nigrociliata*), broader valva, longer tegumen, and thicker and shorter uncus arms. The general shape of the new species' genitalia is somewhat reminiscent of some *Epargyreus* Hübner, [1819] (type species *Papilio tityrus* Fabricius, 1775, a junior homonym, considered a subjective synonym of *Papilio clarus* Cramer, 1775). While this new species is diagnosed by male genitalia, due to unexplored individual variation and other undescribed species, most reliable identification is achieved by DNA, and a combination of the following base pairs is diagnostic in the nuclear genome: aly10226.35.2:A171G, aly10226.35.2:G180A, aly10226.35.2:G186T, aly6654.9.15:G549A, aly6654.9.15:G558A, aly2633.9.6:C30C (not T), aly173.58.7:A72A (not G), aly1205.1.8:C81C (not T), aly1205.1.8:C93C (not T), aly173.58.7:G63G (not A), and COI barcode: T19T (not C), 205T, T208T (not G), T286C, T472C, C517C (not T).

Barcode sequence of the holotype. Sample NVG-24074H03, GenBank PV408305, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACCTCATTAAGATTACTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAATTCCTCTTATACTAGGAGCCCCCGATATAGCTTTCCCTCGTA
TAAATAATATAAGATTTTGATTATTACCCCCATCCTTAACTCTCTTAATTTCAAGAAGAATTGTAGAAAACGGTGCAGGTACTGGATGAACAGTTTATCCCCCTTTATCATCCAATATTGC
CCATCAAGGGGCATCAGTAGATTTAGCAATTTTTTCATTACATCTAGCTGGGATTTCCTCTATTCTTGGAGCTATTAATTTTATTACAACTATTATTAATATACGAATCAATAATTTATCA
TTTGACCAAATACCATTATTTATTTGAGCTGTCGGAATTACAGCTTTATTATTATTACTTTCATTACCTGTTTTAGCAGGAGCTATTACCATATTATTAACTGATCGAAATTTAAATACTT
CTTTTTTTGATCCAGCAGGTGGGGGTGATCCTATTTTATATCAACATTTATTT

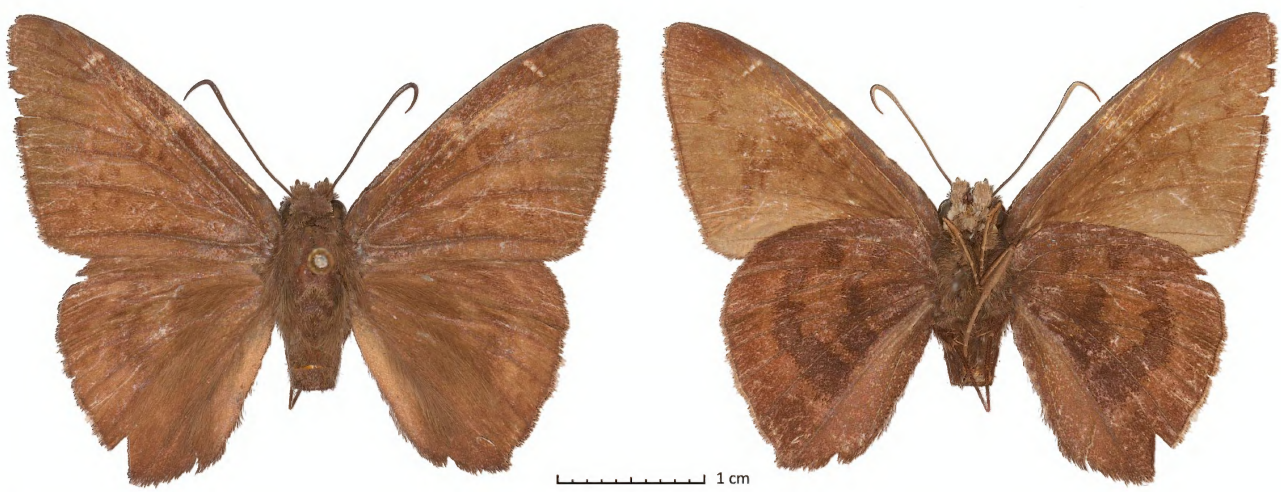

Fig. 4. *Cecropterus* (*Murgaria*) *roeveri* **sp. n.** holotype ♂ NVG-24074H03 in dorsal (left) and ventral (right) views, data in text.

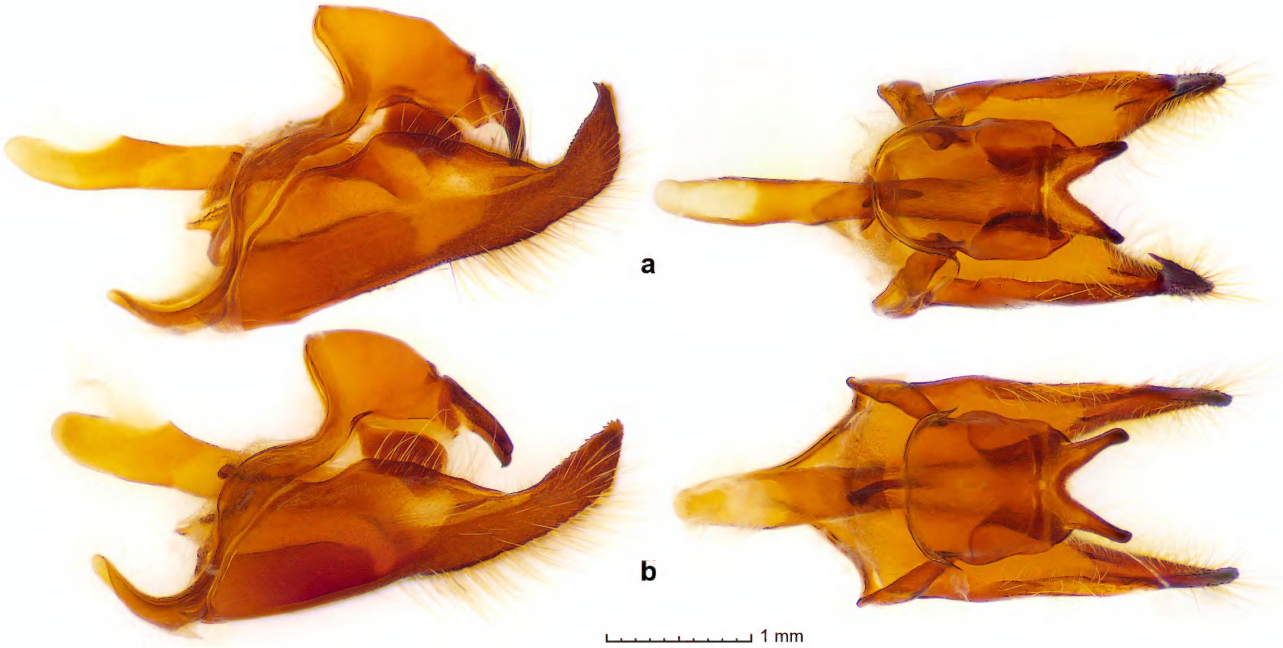

Fig. 5. Male genitalia of *Cecropterus* (*Murgaria*) in left lateral (left) and dorsal (right) views: **a)** *C. roeveri* **sp. n.** holotype NVG-24074H03; **b)** *C. nigrociliata* NVG-24064F03 Mexico: Colima, Comala, 14-Nov-1982, D. W. Jenkins leg. [MGCL].

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 4 (genitalia Fig. 5a), bears the following six printed (text in italics handwritten) rectangular labels, five white: [Arivaca Creek, 3600' | ~~at~~ Arivaca, Pima Co.| AZ ↑ *7 mi. W. of*], [*22* Sept.19*84* | leg. K. Roever], [MGCL Collection | W. McGuire coll. | Ex. K. Roever], [DNA sample ID: | NVG-24074H03 | c/o Nick V. Grishin], [genitalia | NVG241111-34 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Cecropterus (Murgaria) | roeveri Grishin].

Type locality. USA: Arizona, Pima Co., 7 mi west of Arivaca, Arivaca Creek, elevation 3600'.

Etymology. The name, a noun in the genitive case, honors Kilian Roever, the collector of the holotype of this new species and numerous other butterfly records. Kilian's deep passion for nature exploration, extensive knowledge, and countless hours in the field were instrumental in discovering all these rarely observed species in Arizona and beyond, significantly enhancing our understanding of them. Without his immense collecting efforts and excellent specimen preservation, which ensured high-quality DNA, this and many other studies would have been impossible. Kilian eagerly shares stories of his field experiences and generously provides details about the localities of his fascinating discoveries, for which we are deeply grateful. His generosity has enriched us all.

Distribution. Currently known only from the holotype collected in southeastern Arizona, expected in western Mexico.

Spicauda procne (Plötz, 1881) in Arizona and New Mexico

Genomic analysis of several *Spicauda* Grishin, 2019 (type species *Goniurus procne* Plötz, 1881) specimens collected in SE Arizona, and SW New Mexico by Kilian Roever confirms their identification as *Spicauda procne* (Plötz, 1881) (type in Brazil) (Fig. 1 cyan, shaded yellow). The following five specimens were sequenced: 1♂ NVG-24074E08 New Mexico, Hidalgo Co., Peloncillo Mts., Cottonwood Canyon, 0-1.5 mi E of AZ state line, 23-Sep-1990 (Fig. 6) and Arizona, Santa Cruz Co.: 2♀♀ NVG-24087B10 and NVG-24087B11 Atascosa Mts., Sycamore Canyon, 27-Sep-1959; 1♂ NVG-24087C01 3 mi SW of Sonoita, Cottonwood Spring, 5-Oct-1969; and 1♂ NVG-24087B12 O'Donnell Canyon at Ewing

Fig. 6. *Spicauda procne* ♂ NVG-24074E08 from USA, New Mexico in dorsal (left) and ventral (right) views, data in text.

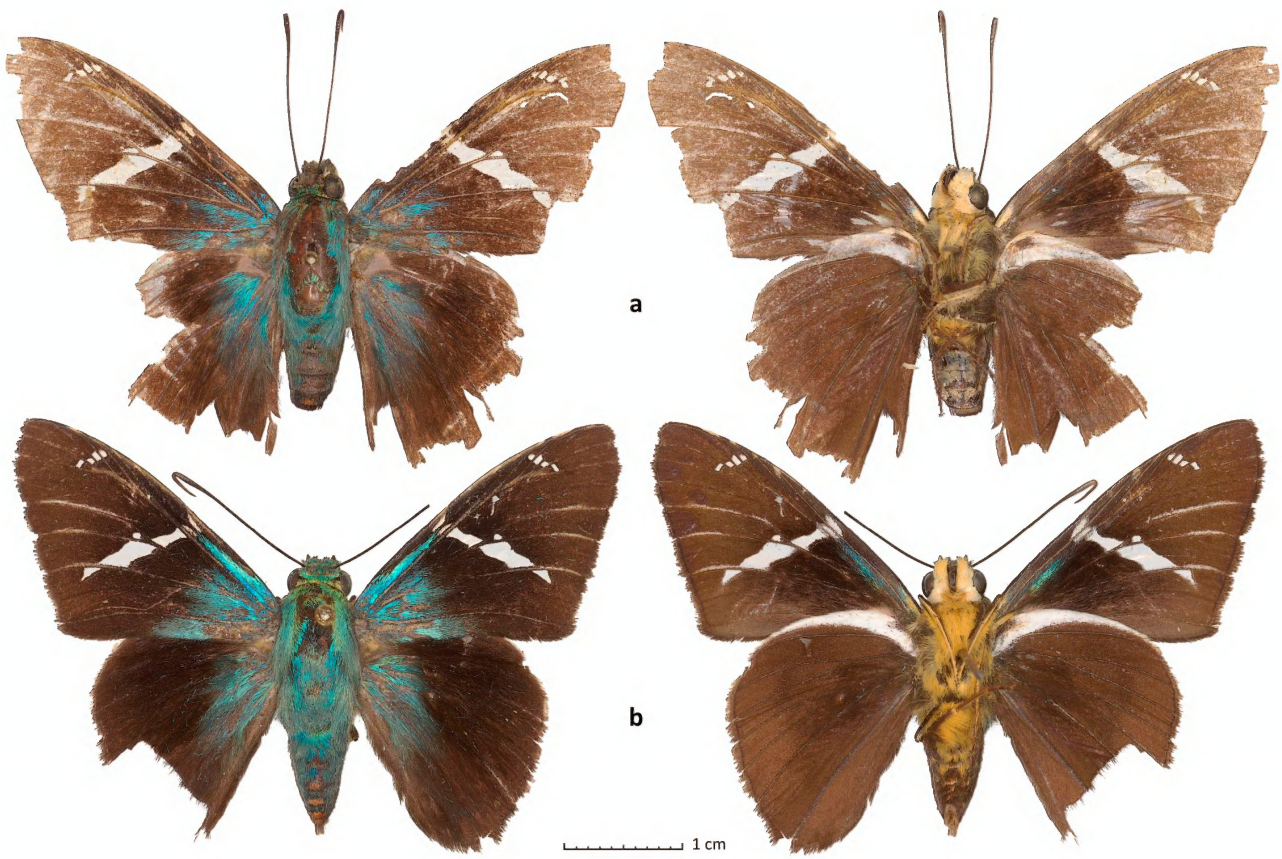

Fig. 7. *Telegonus* (*Telegonus*) *misitra* ♂♂ from USA: Arizona in dorsal (left) and ventral (right) views, data in text: **a)** NVG-24087C03 and **b)** NVG-24087B09.

Ranch, nr. Canelo, 2-Sep-1973. None of the Arizona specimens we sequenced was *Spicauda simplicius* (Stoll, 1790) (type locality in Suriname). However, we confirm *S. simplicius* from Texas, Cameron Co., Brownsville [USNM]: 1♂ NVG-19121D01, USNMENT 01602723 22-Jul-1922, E. M. Smith leg, G. W. Rawson collection and 1♀ NVG-19121C12, USNMENT 01201575 8-Jun-1944 from J. C. Hopfinger collection (Fig. 1 olive, shaded green).

Telegonus (*Telegonus*) *misitra* (Plötz, 1881)

Genomic analysis of two males from the *fulgerator* species group of *Telegonus* Hübner, [1819] (type species *Papilio talus* Cramer, 1777) collected by Kilian Roever in Arizona, Santa Cruz Co., Atascosa Mts., Sycamore Canyon (NVG-24087C03 8-May-1960 (Fig. 7a) and NVG-24087B09 27-Aug-1983 (Fig. 7b)) (Fig. 8 shaded yellow) reveals that they are not *Telegonus* (*Telegonus*) *tsongae* Grishin, 2023 (type locality in USA: Texas, Starr Co.) (Fig. 8 blue), the only species from the group previously known from the US (Fig. 8 shaded green), but *Telegonus* (*Telegonus*) *misitra* (Plötz, 1881) (type locality in Mexico) (Fig. 8 red) instead, because they are in the same clade with its lectotype. Thus, these specimens represent the first US records of *T. misitra*, which was known from Mexico and Costa Rica.

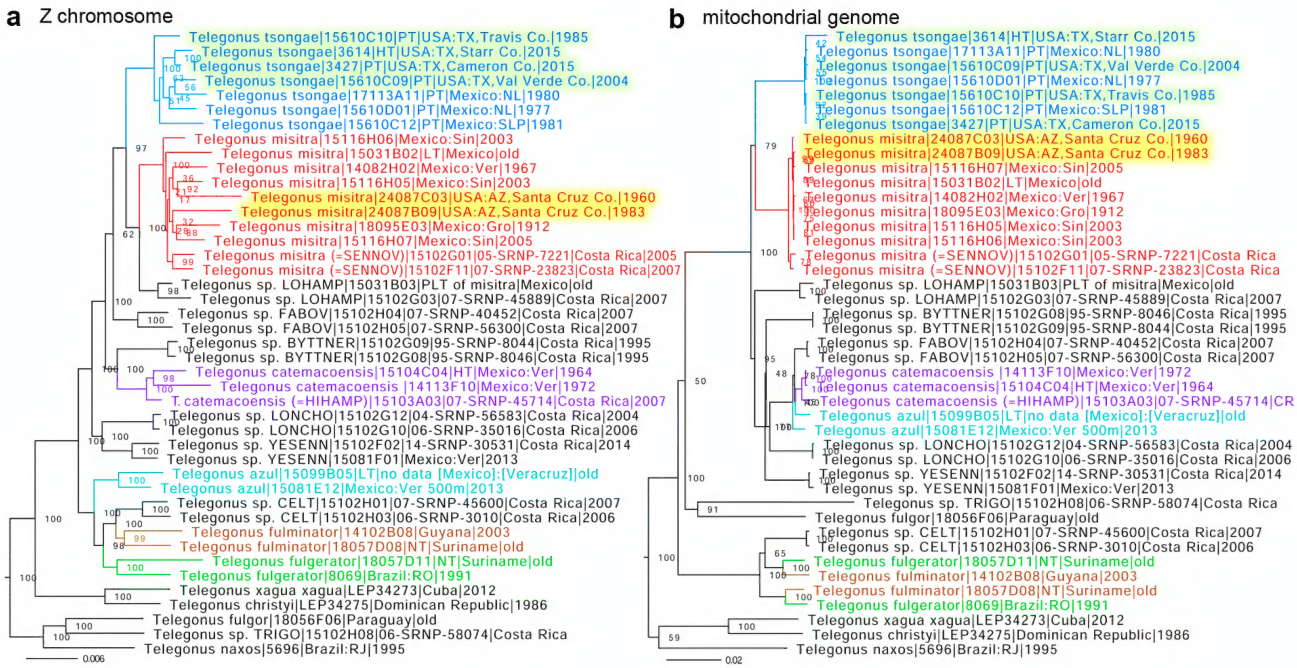

Fig. 8. Phylogenetic trees of selected *Telegonus* (*Telegonus*) species of the *T. fulgerator* group inferred from protein-coding regions in **a)** the Z chromosome, based on 258,849 positions, and **c)** the mitochondrial genome: *T. tsongae* (blue), *T. misitra* (red), *T. catemacoensis* (purple), *T. azul* (cyan), *T. fulminator* (brown), and *T. fulgerator* (green). Labels of specimens from Arizona and Texas are highlighted in yellow and green, respectively. Note rampant mitochondrial genome introgression that precludes confident species identification using COI barcodes. See Fig. 1 caption for other notations.

Epargyreus cruza Evans, 1952

Genomic analysis of a male (NVG-24074H05) (Fig. 9a, 10) collected by Kilian Roever in Arizona, Santa Cruz Co., Peña Blanca Canyon on 2-Apr-1960 (Fig. 11 shaded yellow) reveals that it is not *Epargyreus fractigutta* Grishin, 2023 (type locality USA: Texas, Hidalgo County, McAllen) (Fig. 9b), the only representative of Mexican and Central American species of *Epargyreus* Hübner, [1819] confirmed from the US (Fig. 11 blue, shaded green), but *Epargyreus cruza* Evans, 1952 (type locality Mexico: Veracruz, Cordova) instead, in agreement with its wing patterns (Fig. 9). The differences in wing patterns of these

species are described and illustrated in Zhang et al. (2023b). For instance, the semihyaline spot in the forewing cell CuA_2-1A+2A is offset farther distad from spot in the cell CuA_1-CuA_2 in *E. fractigutta* (Fig. 9b) than in *E. cruza* (Fig. 9a). Whitish lavender overscaling on ventral hindwing in *E. cruza* (Fig. 9a) is better developed between the discal silver spots and the postdiscal white band. However, in E. *fractigutta* (Fig. 9b), the overscaling is better developed between the postdiscal while band and marginal pale-lavender area, which are frequently merged with each other as a result. Due to the potential presence of undescribed species, these phenotypic characters should be used with caution, with genome-based identification providing the highest level of confidence. Thus, this Roever's specimen represents the first

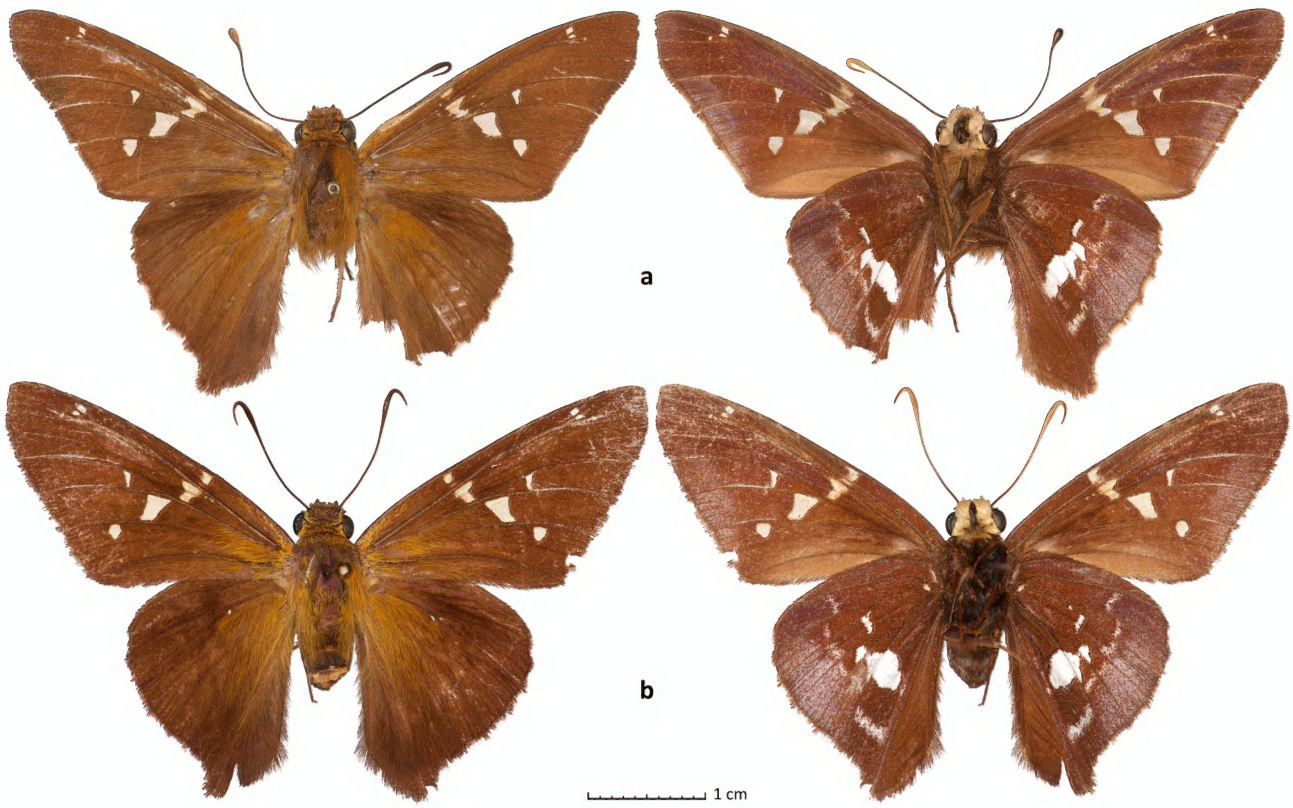

Fig. 9. Specimens of *Epargyreus* from the USA in dorsal (left) and ventral (right) views, data in text: **a)** *E. cruza* ♂ NVG-24074H05 from Arizona; **b)** *E. fractigutta* holotype ♀ NVG-14111F08 from Texas.

Fig. 10. Male genitalia of *Epargyreus cruza* NVG-24074H05 from USA: Arizona in left lateral (left) and dorsal (right) views.

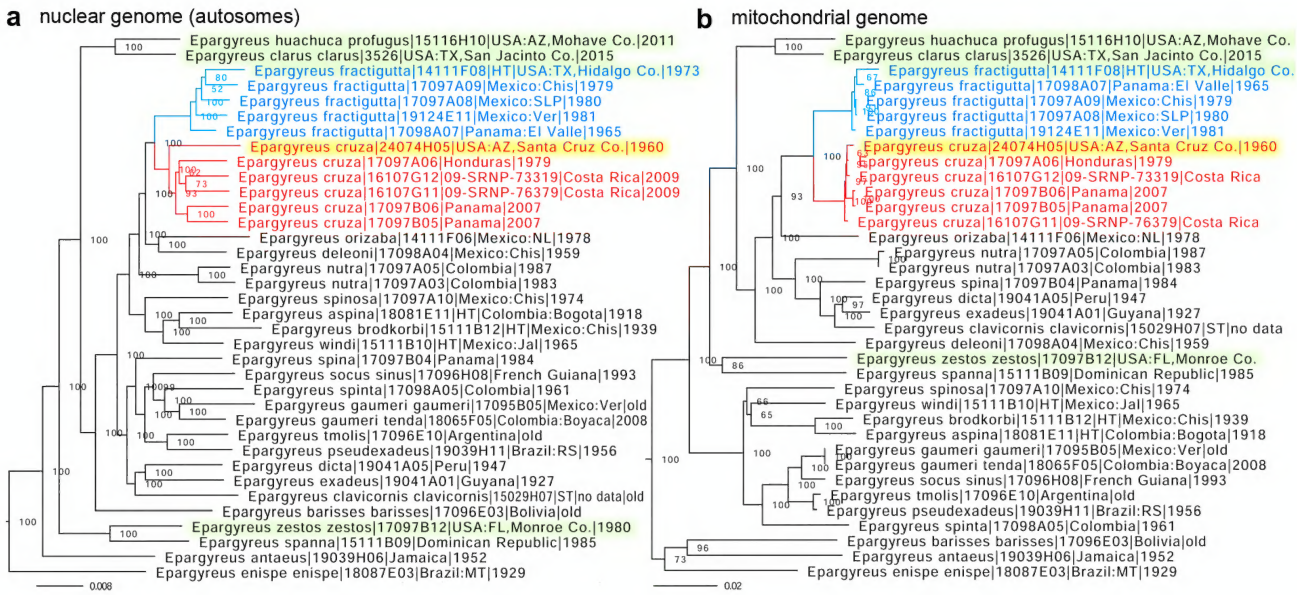

Fig. 11. Phylogenetic trees of selected *Epargyreus* species inferred from protein-coding regions in **a)** the nuclear genome (autosomes), based on 4,239,540 positions, and **b)** the mitochondrial genome: *E. fractigutta* (blue) and *E. cruza* (red). Labels of a specimen from Arizona collected by Roever and other USA specimens are highlighted in yellow and green, respectively. See Fig. 1 caption for other notations.

US record of *E. cruza*, a name previously applied to *E. fractigutta* because of misidentification. We are not aware of *E. cruza* records from Texas.

Ectomis (*Asina*) *mexicanus* (H. Freeman, 1969)

Genomic analysis of a male (NVG-24074E05) (Fig. 12) collected by B. Griffin (Bailowitz and Brock 1991, 2022) in Arizona, Pima Co., Box Canyon on 4-Oct-1967 confirms its identification as *Ectomis* (*Asina*) *mexicanus* (H. Freeman, 1969) (type locality in Mexico: San Luis Potosi) (Fig. 13 red, shaded yellow). This species has also been recorded from Texas (Fig. 13 red, shaded green).

Fig. 12. *Ectomis* (*Asina*) *mexicanus* ♂ NVG-24074E05 from Arizona in dorsal (left) and ventral (right) views, data in text.

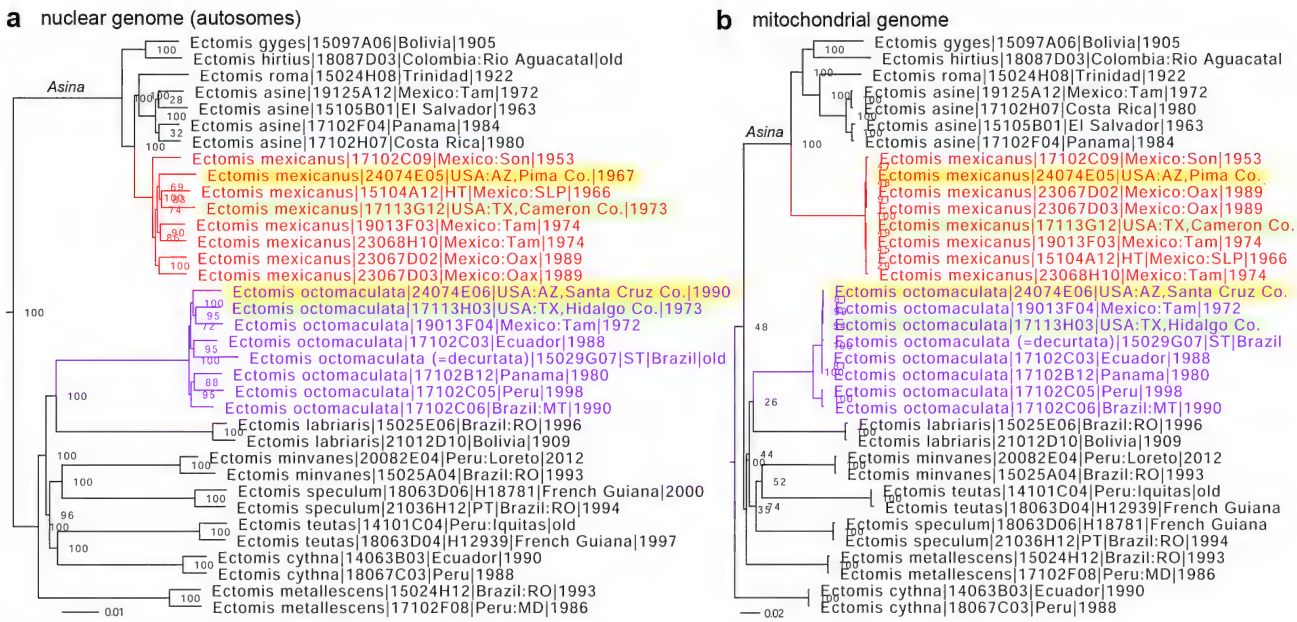

Fig. 13. Phylogenetic trees of selected *Ectomis* species inferred from protein-coding regions in **a)** the nuclear genome (autosomes), based on 4,043,244 positions, and **b)** the mitochondrial genome: *E.* (*Asina*) *mexicanus* (red) and *Ectomis* (*Ectomis*) *octomaculata* (purple). Labels of specimens from Arizona and Texas are highlighted in yellow and green, respectively. The clade corresponding to the subgenus *Asina* Grishin, 2019 (type species *Eudamus asine* Hewitson, 1867) is labeled. See Fig. 1 caption for other notations.

Ectomis (*Ectomis*) *octomaculata* (Sepp, [1844])

Genomic analysis of a male (NVG-24074E06) (Fig. 14) collected by Kilian Roever in Arizona, Santa Cruz Co., Santa Rita Mts., Temporal Gulch on 29-Sep-1990 confirms its identification as *Ectomis* (*Ectomis*) *octomaculata* (Sepp, [1844]) (type locality in Suriname) (Fig. 13 purple, shaded yellow). This species is also known from Texas (Fig. 13 purple, shaded green).

Fig. 14. *Ectomis* (*Ectomis*) *octomaculata* ♂ NVG-24074E06 from AZ in dorsal (left) and ventral (right) views, data in text.

Celaenorrhinus fritzgaertneri (Bailey, 1880)

Genomic analysis of a specimen (NVG-24074F03) (Fig. 15) collected by Kilian Roever in Arizona, Cochise Co., Peloncillo Mts., Cottonwood Canyon, 4800-5200' on 23-Aug-1983 confirms its

identification as *Celaenorrhinus fritzgaertneri* (Bailey, 1880) (type locality in El Salvador) (Fig. 16 shaded yellow). Both *C. fritzgaertneri* and *Celaenorrhinus stallingsi* H. Freeman, 1946 (type locality Mexico: Nuevo Leon, Monterrey) are confirmed from Texas by genomic sequencing (Fig. 16 shaded green).

Fig. 15. *Celaenorrhinus fritzgaertneri* NVG-24074F03 from USA: Arizona in dorsal (left) and ventral (right) views, data in text.

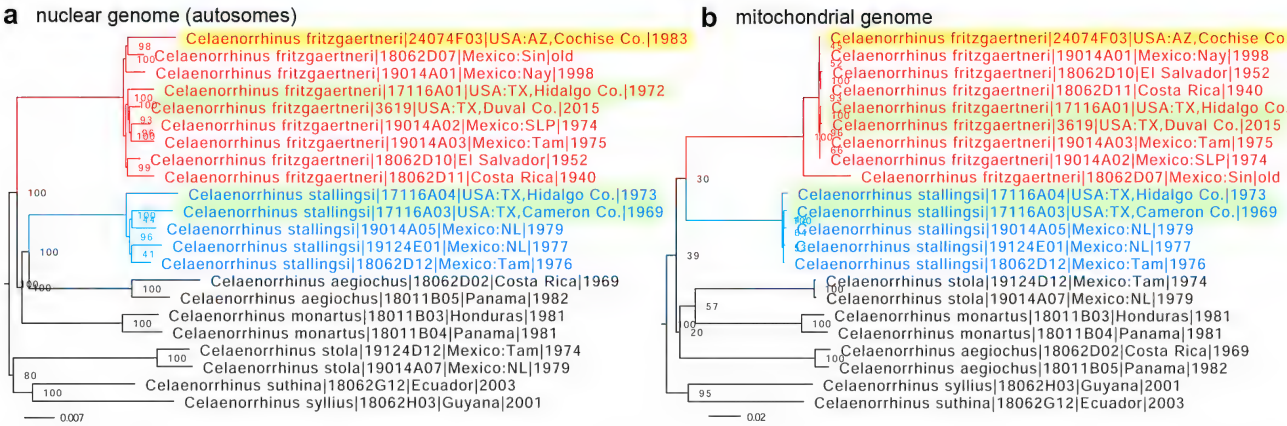

Fig. 16. Phylogenetic trees of selected *Celaenorrhinus* species inferred from protein-coding regions in **a)** the nuclear genome (autosomes), based on 2,246,907 positions, and **b)** the mitochondrial genome: *C. fritzgaertneri* (red) and *C. stallingsi* (blue). Labels of specimens from Arizona and Texas are highlighted in yellow and green, respectively. See Fig. 1 caption for other notations.

Lerema (*Lerema*) *ochrius occidus* Grishin, new subspecies

http://zoobank.org/E9489DAF-BB97-4F6A-9372-68B9B2CA4D91

(Figs. 17 part, 18)

Definition and diagnosis. Closely related to *Lerema* (*Lerema*) *ochrius* Grishin, 2023 (type locality in USA: Texas, Hidalgo Co.) this new subspecies is genetically differentiated from it and corresponds to a separate clade in the Z chromosome and mitochondrial genome trees (Fig. 17), while differing by 1.5% (10 bp) in the COI barcode. Due to somewhat limited genetic differentiation and phenotypic similarity, this taxon is proposed as a subspecies. This new subspecies keys to *Lerema accius accius* (J. E. Smith, 1797) (type locality in USA: Georgia) (J.39.2(a)) in Evans (1955), and is more similar to *L. ochrius* in less reddish-brown and more ochreous-brown tones of ventral side of wings, but differs from it by less olive and more brown tones; being paler and more washed out on the ventral surface with larger and blotchier hindwing pale spots and patches (especially the discal area basad of the postdiscal band); and

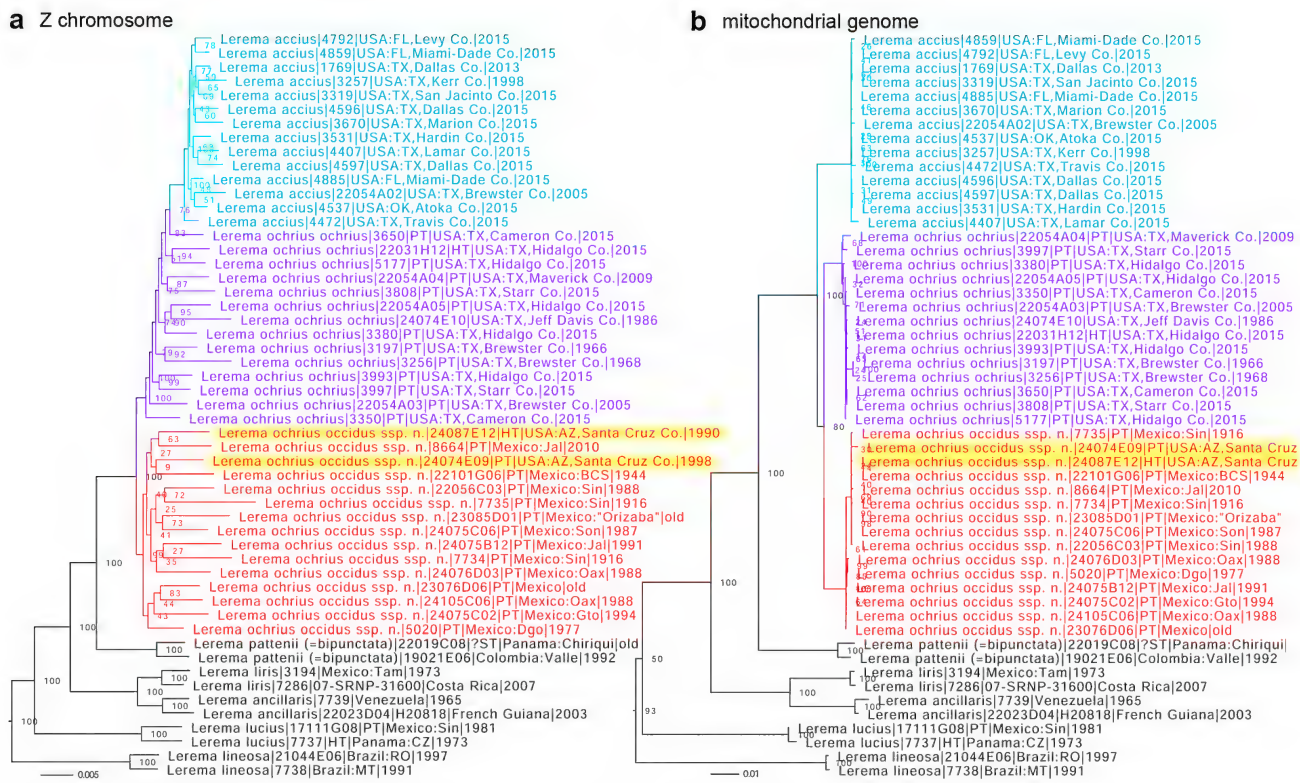

Fig. 17. Phylogenetic trees of selected *Lerema* (*Lerema*) species inferred from protein-coding regions in **a)** the Z chromosome, based on 187,050 positions, and **b)** the mitochondrial genome: *Lerema* (*Lerema*) *accius* (blue), *Lerema* (*Lerema*) *ochrius ochrius* (purple), and *Lerema* (*Lerema*) *ochrius occidus* **ssp. n.** (red, labels of AZ specimens highlighted in yellow). See Fig. 1 caption for other notations.

females with larger forewing semihyaline hyaline spots. Due to the cryptic nature of this subspecies, most reliable identification is achieved by DNA, and a combination of the following base pairs is diagnostic in the nuclear genome: aly276558.4.10:G749A, aly276558.4.10:G844A, aly276558.4.10:T897C, aly276558.4.10:C936T, aly276558.4.10:C1458T, and COI barcode: A100G, A214G, T247T (not C), C274C (not T), T340C, T644C.

Barcode sequence of the holotype. Sample NVG-24087E12, GenBank PV408306, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATATTAGGAACTTCTTTAAGCTTATTAATTCGAACAGAATTAGGAAATCCAGGATCTTTAATTGGGGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTCCCATTAATACTGGGAGCTCCTGATATAGCATTTCCACGAA
TAAATAACATAAGATTTTGAATATTACCTCCCTCATTAATACTATTAATTTCAAGTAGAATTGTAGAAAATGGTGCAGGAACAGGATGAACAGTTTACCCACCTTTATCCTCTAATATTGC
CCATCAAGGAGCATCAGTTGATTTAGCAATTTTTTCTCTTCATTTAGCAGGTATTTCTTCAATTTTAGGAGCTATTAATTTTATTACTACAATTATTAATATACGAGTTAGAAATTTATCT
TTTGATCAAATACCTTTATTTGTTTGATCTGTCGGAATTACAGCATTATTATTACTCTCACTACCTGTATTAGCTGGAGCTATTACTATACTTTTAACTGATCGAAATCTTAATACTT
CTTTTTTTGATCCTGCAGGAGGTGGTGATCCTATTTTATACCAACATTTATTT

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 18a, bears the following five printed (text in italics handwritten) rectangular labels, four white: [AZ: Santa Cruz Co., | Lake Patagonia S.P.], [*8* Sept.19*90* | leg. Kilian Roever], [MGCL collection | W. McGuire Coll. | Ex K. Roever], [DNA sample ID: | NVG-24087E12 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Lerema (Lerema) ochrius | occidus Grishin]. **Paratypes:** 9♂♂ and 5♀♀: 1♂ NVG-24074E09 USA, Arizona, Santa Cruz Co., O'Donnell Canyon at Ewing Ranch nr. Canelo, 23-Aug-1998, Kilian Roever leg. [MGCL] and from Mexico: 1♂ NVG-23076D06 no location details, from Herrich-Schäffer collection, before 1874 [MFNB]; 1♂ NVG-22101G06, CASENT8566924 Baja California Sur, San Jose del Cabo, Pescadero 22-Mar-1944 [CAS]; 1♂ NVG-24075C06 Sonora, Santa Rosa–Yécora Road, 1–2 mi SE of Santa Rosa, 13-Apr-1987, Jim P. Brock [MGCL]; Sinaloa: 1♀ NVG-22056C03 Hwy 40 El Palmito, 1970 m, 12-Oct-1988, John Kemner leg. [TMMC] and Mazatlan, Dec-1916, J. August Kusche leg. [USNM]: 1♂ NVG-7734, USNMENT 01321574, genitalia NVG170205-19 and 1♀ NVG-7735, USNMENT 01321575, genitalia

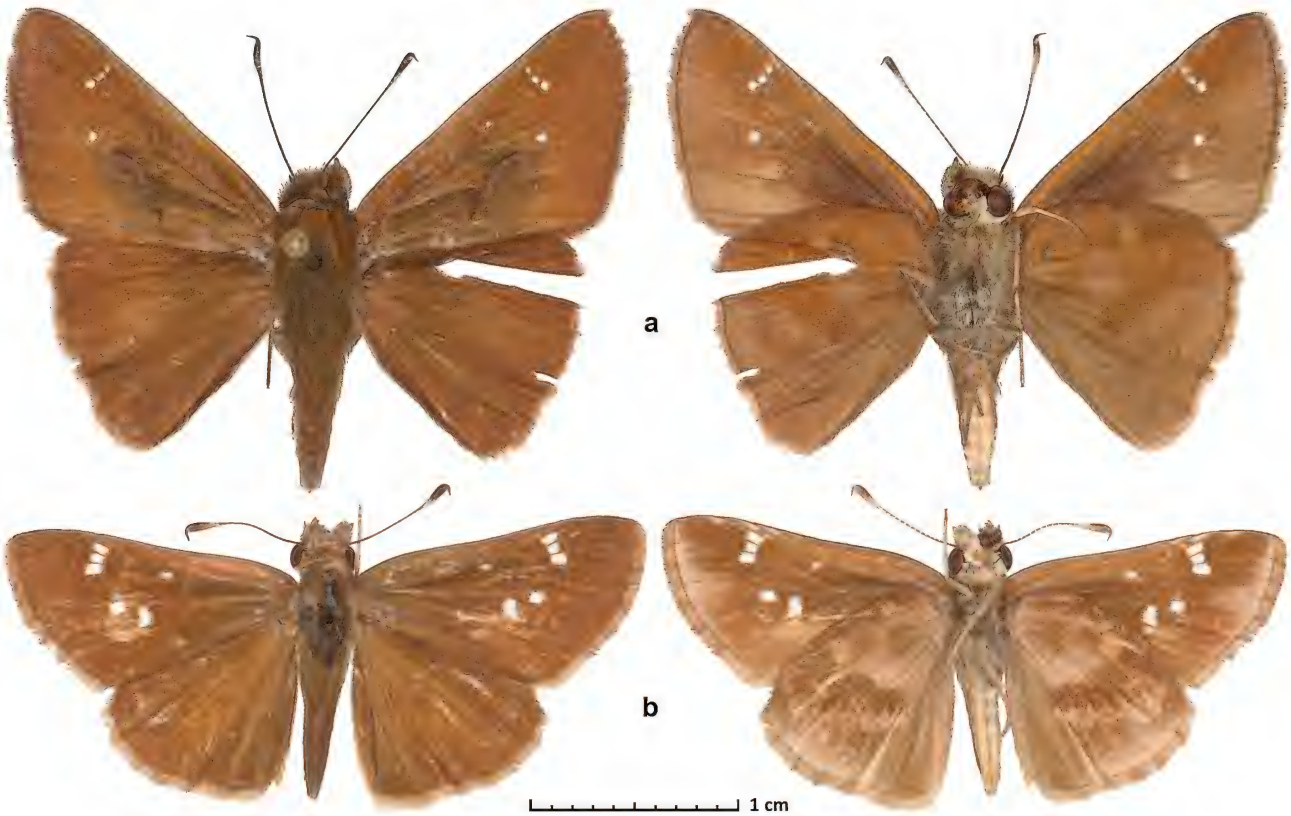

Fig. 18. Type specimens of *Lerema* (*Lerema*) *ochrius occidus* in dorsal (left) and ventral (right) views, data in text: **a)** holotype ♂ NVG-24087E12 from USA: Arizona and **b)** paratype ♀ NVG-7735 from Mexico: Sinaloa.

NVG170205-20 (Fig. 18b); 1♂ NVG-5020 Durango, 8 km W Los Mimbres Creek, 16-Sep-1977, W. W. McGuire leg., genitalia NVG151101-71 [USNM]; Jalisco: 1♀ NVG-24075B12 La Garita, 20-Nov-1991, D. L. & J. Lindsley leg. [MGCL] and 1♀ NVG-8664 Hesp-EB 02 424 San Juan de la Montana, 1820 m, GPS 19.67, −103.07, 6-Aug-2010, G. Nogueira leg. [Ernst Brockmann collection]; 1♂ NVG-24075C02 Guanajuato, San Miguel de Allende, 25-Dec-1994, D. L. Lindsley leg. [MGCL]; Oaxaca, Hwy 175, ca. 5 mi N of Oaxaca, J. Kemner leg.: 1♂ NVG-24105C06 11-Aug-1988 [TMMC] and 1♀ NVG-24076D03 22-Jul-1988 [MGCL]; and 1♂ NVG-23085D01 "Mexico, Orizaba, ex coll. Neuburger" old, likely mislabeled [ZSMC].

Type locality. USA: Arizona, Santa Cruz Co., Patagonia Lake State Park.

Etymology. In Latin, *occidens* means west or western, forming the basis of the name, which is treated as a noun in apposition.

Distribution. From the southwestern US through western to southern Mexico.

Comment. While conservatively proposed here as a subspecies pending further research, it may be a species-level taxon.

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